

THE DISTRIBUTION OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE “ECSTASY”-INDUCED *C-FOS* EXPRESSION IN RAT BRAIN

C. P. STEPHENSON,* G. E. HUNT,† A. N. TOPPLE*† and I. S. MCGREGOR*‡

*Department of Psychology, University of Sydney, A19, Sydney, NSW 2006, Australia

†Department of Psychological Medicine, University of Sydney Clinical Sciences Building, Concord Hospital, Concord, NSW 2139, Australia

Abstract—Rats were injected with 3,4-methylenedioxymethamphetamine (“Ecstasy”) and assessed for changes in locomotor activity and for the expression of the immediate early gene *c-fos* throughout the brain. A dose-dependent increase in locomotor activity was seen with 3,4-methylenedioxymethamphetamine (0, 5 and 20 mg/kg) that continued for at least 2 h following administration. Dose-dependent increases in *c-fos* expression were seen in much of the cortex, forebrain, brainstem and cerebellum in rats given 3,4-methylenedioxymethamphetamine. Expression was pronounced in 5-hydroxytryptamine terminal regions including the medial prefrontal cortex, caudate–putamen, nucleus accumbens, olfactory tubercle, islands of Calleja, lateral septum, paraventricular hypothalamus and paraventricular thalamus. High levels of *c-fos* expression were also seen in the supraoptic and median preoptic nuclei, regions involved in the control of fluid balance and body temperature, respectively. This is potentially important since deaths in 3,4-methylenedioxymethamphetamine users have been linked to hyperthermia and hyponatremia. In the brainstem, two regions of high *c-fos* expression were Barrington’s nucleus, which is involved in micturition, and the pontine reticular nucleus oralis, a region involved in motor control of mastication. Activation of this latter structure may partly explain the bruxism (grinding of the jaw) reported by human 3,4-methylenedioxymethamphetamine users. Robust *c-fos* expression was seen in the cerebellum, particularly in the flocculus, and this may explain the reported deleterious effects of 3,4-methylenedioxymethamphetamine on balance and co-ordination. Significant *c-fos* expression was also seen in the ventral tegmental area, amidst the cell bodies of mesolimbic and mesocortical dopamine neurons, and in the median and dorsal raphe, where the serotonergic innervation of the forebrain originates. Double-labelling of *fos*-positive neurons with 5-hydroxytryptamine showed that only a small number of serotonergic neurons in the raphe expressed *c-fos* following 3,4-methylenedioxymethamphetamine.

The widespread distribution of 3,4-methylenedioxymethamphetamine-induced *c-fos* expression seen in this study can be linked to the profound alterations in physiological function, mood and behaviour produced by this drug. © 1999 IBRO. Published by Elsevier Science Ltd.

Key words: MDMA, Ecstasy, 5-HT, dopamine, *c-fos*, rat.

3,4-Methylenedioxymethamphetamine (MDMA; “Ecstasy”) is a popular drug throughout the world, particularly among young adults involved in the “rave” subculture.^{32,76} The past 10 years has seen increasing popularity of MDMA, with users reporting sociably desirable effects including energy, confidence, elevated mood and feelings of closeness towards others.^{46,64,77,83} However, there are also

increasing reports of adverse effects associated with the drug including possible depression,¹⁵ anxiety^{51,53} and cognitive impairment.⁶¹ The drug has also been linked to a small number of deaths among users, usually attributed to drug-induced disruptions of thermoregulatory processes or impairment of fluid and electrolyte balance.^{32,37}

The specific neurochemical effects of MDMA have become increasingly well documented over the past few years. MDMA causes a massive elevation of synaptic 5-hydroxytryptamine (5-HT) via both carrier-mediated release and re-uptake inhibition.^{5,26,75} Although there are only a relatively small number of serotonergic neurons in the brain, the system has extensive axonal arborizations, innervating numerous cortical and limbic regions.³³ By globally enhancing synaptic 5-HT levels, MDMA is able to produce many cognitive, behavioural, emotional, physiological and hormonal effects.

‡To whom correspondence should be addressed.

Abbreviations: ADH, antidiuretic hormone; BNST, bed nucleus of the stria terminalis; CEA, central nucleus of the amygdala; 5-HT, 5-hydroxytryptamine; MDMA, 3,4-methylenedioxymethamphetamine, “Ecstasy”; MPC, medial prefrontal cortex; MPO, median preoptic nucleus; NAS, nucleus accumbens septi; PB, phosphate buffer; PBH, phosphate-buffered horse serum; PBS, phosphate-buffered saline; PCA, *p*-chloroamphetamine; PNO, pontine reticular nucleus oralis; PVN, paraventricular nucleus of the hypothalamus; SON, supraoptic nucleus.

MDMA also increases synaptic levels of dopamine and noradrenaline,^{26,59,85} although these effects are less profound than those on brain 5-HT systems.

The acute enhancement of 5-HT produced by MDMA may be followed by a long-term impairment in 5-HT function. An initial depletion of 5-HT is seen within 4 h of MDMA administration in rats, and subacutely within two to three days.⁷⁵ The later depletion is longer lasting and is accompanied by a number of markers of neurotoxicity in 5-HT neurons, particularly in terminals.^{25,60} Recovery from the neurotoxic effect of MDMA is sometimes seen in rats,⁷³ but has not been observed in monkeys.⁶⁷ Very recent evidence suggests that 5-HT neurotoxicity may also occur with repeated MDMA use in humans.⁵² Considering the importance of 5-HT in the genesis of a number of psychiatric conditions, the permanent rearrangement and destruction of serotonergic neurons by MDMA may potentially predispose susceptible individuals to psychopathology.

The immediate early genes are a group of recently discovered transcription factors of which several appear to play an important role in the regulation of CNS development and function.³⁹ In this large family of over 100 members, the proto-oncogene known as *c-fos* has been extensively investigated due to its early expression following diverse CNS processes and its low constitutive expression.¹⁶ Expression of *c-fos* occurs through a number of different signal transduction pathways which work on distinct upstream regulatory elements including the serum-response element and the calcium/cyclic-adenosine monophosphate response element.³⁹ Expression of *c-fos* in specific neurons often occurs following recent activation thereby allowing an *in vivo* map of cellular responses to a given stimulus. The exact functional significance of *c-fos* is unknown but appears to be involved in coupling extracellular stimuli to long-term cellular changes by regulating the expression of specific target genes.¹⁶

A diverse number of psychoactive drugs cause *c-fos* expression, with a predominant response seen in striatal and limbic structures containing the neurotransmitter and receptor systems that the drug is active upon.³⁴ On the assumption that the regions of *c-fos* expression indicate the neural substrates of drug action, observed behavioural effects of a drug can be correlated with *c-fos* expression in specific brain regions. Consequently *c-fos* immunohistochemistry can be used to integrate knowledge about behaviour, neurotransmitter systems and brain regions to better understand the actions of psychoactive drugs.³⁴

To date, two papers have investigated *c-fos* expression following MDMA administration in rats, noting extensive *c-fos* expression in the frontal cortex, striatum, nucleus accumbens and olfactory tubercle.^{22,35} However, both these studies have

only evaluated *c-fos* expression in a very limited set of forebrain regions. The present study aims to extend these findings by assessing MDMA-induced *c-fos* expression in a wide range of cortical, limbic and brainstem sites. The overall aim of this study is to further illuminate the neural basis of some of the diverse behavioral and physiological actions of MDMA.

EXPERIMENTAL PROCEDURES

Subjects

The study involved a total of 15 outbred male Wistar rats (Gore Hill, Sydney) weighing between 370–470 g at the time of testing. The rats were housed in two large plastic tubs in groups of eight. One rat from one of the cages was not used in the experiment. They were kept in a colony under a 12:12 h reversed light:dark cycle (lights off 9.00 a.m.) with *ad libitum* access to food and water. All experimentation occurred during the dark cycle. All procedures used were approved by the Animal Welfare Committee of the Central Sydney Area Health Service. Every effort was made to use the smallest number of animals possible and to minimize any animal suffering.

Drug

(+)-3,4-Methylenedioxymethamphetamine (MDMA) was generously supplied by the National Institute on Drug Abuse (U.S.A.) as the hydrochloride salt. Two doses were used in the study, 5 mg/kg and 20 mg/kg, dissolved in physiological saline and injected in a volume of 1 ml/kg. The 5 mg/kg dose was chosen since it approximates the typical dose used by humans (1–4 mg/kg), and is associated with clear behavioural and physiological effects in rats.^{17,78} The higher 20 mg/kg dose, exerts greater behavioural and neurochemical effects in rats than the 5 mg/kg dose^{43,54} and is known to cause a long-term neurotoxic effect on brain 5-HT systems.⁷⁴

Procedure

Rats were randomly assigned to one of three groups ($n=5/\text{group}$) to receive either MDMA (20 mg/kg), MDMA (5 mg/kg) or vehicle on test day. The assignment was such that the groups were equivalent in baseline locomotor activity and body weight.

The rats were extensively handled for a week prior to the start of experimentation. Two days of familiarization to the test procedure were given immediately prior to the actual test day in order to minimize any *c-fos* expression due to novelty of testing, handling or injection procedures. On these familiarization days the rats were subjected to exactly the same behavioural testing procedures to those used on the test day except that the rats were given only saline injections.

The testing procedures were as follows. Individual rats were removed from their home cage and brought through to the test room in a small enclosed rectangular tub filled with wood shavings. Each rat was then given a single i.p. injection of either vehicle (on habituation days) or MDMA or vehicle (on test day). Exactly 1 min after injection each rat was placed in one of eight identical operant chambers (30×50×25.5 cm). The side walls and roof of the chambers were aluminium, while the front wall and rear wall were made of clear perspex. The floor of each chamber consisted of 16 steel rods. Each chamber was housed inside a light and sound attenuating wooden box (69×71×61.5 cm) with ventilation fans which provided background masking noise.

Table 1. Fos-immunoreactive cells (mean $\pm$ SEM) per 0.5 mm² in various brain regions after saline or 3,4-methylenedioxymethamphetamine "Ecstasy" ($n = 5/\text{group}$)

	Area	Bregma (mm)	Saline	MDMA 5 mg/kg	MDMA 20 mg/kg
1	Medial prefrontal cortex	+ 3.70	7 $\pm$ 2	22 $\pm$ 2.5*	44 $\pm$ 6.3 [‡]
2	Anterior olfactory nucleus (medial)	+ 3.70	5 $\pm$ 2.2	20 $\pm$ 3.1*	36 $\pm$ 4.7 [‡]
3	Nucleus accumbens (core)	+ 2.70	2 $\pm$ 0.5	14 $\pm$ 3.0*	47 $\pm$ 7.0 [‡]
4	Nucleus accumbens (shell)	+ 0.70	2 $\pm$ 1	6 $\pm$ 3.8	19 $\pm$ 3.1 [‡]
5	Islands of Calleja (major)	+ 0.70	1 $\pm$ 0.5	31 $\pm$ 14	49 $\pm$ 14 [‡]
6	Lateral septal nucleus (ventral)	+ 0.70	2 $\pm$ 0.6	14 $\pm$ 2.8*	45 $\pm$ 10 [‡]
7	Caudate-putamen (medial)	+ 0.70	0 $\pm$ 0	20 $\pm$ 8.0*	69 $\pm$ 6 [‡]
8	Caudate-putamen (central)	+ 0.70	0 $\pm$ 0	11 $\pm$ 5.3	46 $\pm$ 11 [‡]
9	Caudate-putamen (lateral)	+ 0.70	0 $\pm$ 0	3 $\pm$ 1.0	16 $\pm$ 4.3 [‡]
10	Olfactory tubercle	- 0.26	0.2 $\pm$ 0.2	8 $\pm$ 2.4	36 $\pm$ 7.7 [‡]
11	Bed nucleus of the stria terminalis	- 0.26	2 $\pm$ 0.5	12 $\pm$ 2.3*	32 $\pm$ 4.2 [‡]
12	Median preoptic nucleus	- 0.26	1 $\pm$ 0.4	7 $\pm$ 1.8	24 $\pm$ 3.8 [‡]
13	Paraventricular nucleus of the hypothalamus	- 1.80	6 $\pm$ 3.0	19 $\pm$ 6.1	39 $\pm$ 14 [‡]
14	Supraoptic nucleus	- 1.80	0 $\pm$ 0	5 $\pm$ 1.5	35 $\pm$ 9 [‡]
15	Central nucleus of the amygdala	- 2.80	5 $\pm$ 2.9	46 $\pm$ 5.6*	49 $\pm$ 11*
16	Paraventricular nucleus of the thalamus	- 2.80	7 $\pm$ 1.4	47 $\pm$ 7.5*	62 $\pm$ 9.2*
17	Edinger - Westphal nucleus	- 5.80	2 $\pm$ 1.1	14 $\pm$ 4.5	31 $\pm$ 7.3 [‡]
18	Ventral tegmental area	- 6.30	0.6 $\pm$ 0.4	5 $\pm$ 2.0	14 $\pm$ 3.9 [‡]
19	Barrington's nucleus	- 6.98	1 $\pm$ 0.4	4 $\pm$ 1.8	31 $\pm$ 7.1 [‡]
20	Pontine reticular nucleus oralis	- 8.00	3 $\pm$ 1.2	8 $\pm$ 2.0	32 $\pm$ 4.1 [‡]
21	Dorsal raphe nucleus	- 7.64	1 $\pm$ 0.6	9 $\pm$ 1.7*	20 $\pm$ 3.3 [‡]
22	Median raphe nucleus	- 7.64	0 $\pm$ 0	2 $\pm$ 0.9	7 $\pm$ 1.2 [‡]
23	Flocculus of the cerebellum	- 10.04	2 $\pm$ 0.9	94 $\pm$ 27.0	181 $\pm$ 42.3 [‡]
24	Lobule 1 of the cerebellum	- 10.04	0 $\pm$ 0	6 $\pm$ 6.0	106 $\pm$ 29.9 [‡]
25	Raphe magnus nucleus	- 10.30	0.2 $\pm$ 0.2	0.8 $\pm$ 0.4	10 $\pm$ 0.5 [‡]

One-way ANOVA, d.f. (2,12), with Tukey's post hoc test: * $P < 0.05$ relative to saline group, [‡] $P < 0.05$ relative to 5 mg/kg MDMA group.

All cell counts per 0.5 mm² except for islands of Calleja, median preoptic nucleus and supraoptic nucleus which were counted per 0.25 mm².

Locomotor activity was assessed by two passive infra-red motion detectors (Jaytech, Sydney) located in each cage. The detectors were located at ground level, one in the centre of the left wall and the second directly opposite this in the right wall and were customized so that they triggered whenever the rat moved in the chamber. The detectors were sensitive to relatively small movements of the head and body of a rat as well as gross locomotion. The output of the detectors was sent to a Macintosh computer running WorkbenchMac software which derived the total number of seconds spent moving in each minute of the 2 h test session.

Immediately after the activity test, the rats were returned to the holding cage. On the two familiarization days the rats were then returned to the colony room. On the test day, the rats were removed to a laboratory where they were given a lethal overdose of pentobarbitone (120 mg/kg, i.p.) and then perfused.

A 2 h interval between drug injection and perfusion was used since a previous report indicated that striatal *c-fos* expression is maximal at 2 h following MDMA.²²

Immunohistochemistry

Immunohistochemical procedures were as described previously.^{40,81} Rats were perfused transcardially with 100 ml of 0.1 M phosphate-buffered saline (PBS) followed by 200 ml of 4% paraformaldehyde in PBS (pH 7.3). The brains were removed and placed in paraformaldehyde overnight at 4°C and stored in cold 15% sucrose for 24 h followed by 30% sucrose for 48 h. The brains were then

placed on microtome stages, frozen to -17°C and sliced at 40 μ m in the coronal plane. Sections were collected in vials containing PBS.

Free-floating sections were incubated for 30 min in 1% H₂O₂ in phosphate buffer (PB) and then for 30 min in 3% normal horse serum in PB. The sections were then incubated in the primary *c-fos* antibody for 72 h at 4°C (Santa Cruz Biotechnology; rabbit polyclonal; specific for the amino terminus of *c-fos* p62, non-cross-reactive with FosB, Fra-1 or Fra-2). The primary antibody was diluted 1:2000 in phosphate buffered horse serum (PBH) (0.1% bovine serum albumin, 0.2% Triton X-100, 2% normal horse serum in PB). Sections were then washed for 30 min in PB and incubated in the secondary antibody (Vector Laboratories; biotinylated anti-rabbit IgG; diluted 1:500 in PBH) at room temperature for 1 h. They were then washed in PB for a further 30 min and then incubated for 1 h in ExtrAvidin-horseradish peroxidase (Sigma; diluted 1:1000 in PBH). After three 30 min washes in PB, horseradish peroxidase activity was visualized with the nickel diaminobenzidine and glucose oxidase reaction. This reaction was terminated after approximately 10 min by washing in PB. The sections were then mounted onto subbed slides, dehydrated, xylene cleared and coverslipped.

Some tissue was processed in a double-labelling procedure⁶³ to allow visualization of 5-HT in addition to the Fos protein. After processing for *c-fos*, as described above, the tissue was washed three times for 30 min in PB and then incubated for 30 min in 1% H₂O₂, and then in 3% normal horse serum in PB. The tissue was then incubated overnight

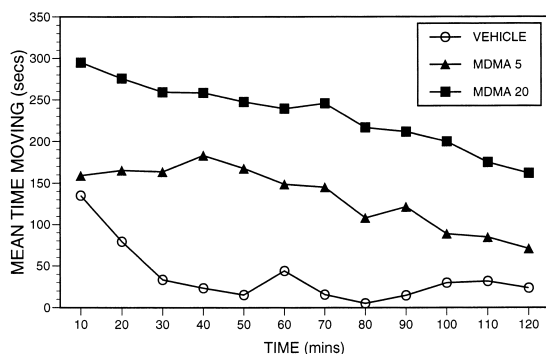


Fig. 1. Locomotor activity in the 2 h following injection of vehicle, 5 mg/kg or 20 mg/kg MDMA. The mean number of seconds spent moving in each 10 min bin over the 2 h period is shown.

at 4°C in the primary 5-HT antibody (Incstar Corporation; rabbit polyclonal; diluted 1:20,000 in PBH). The sections were then washed in PB and incubated in the secondary antibody (Vector Laboratories; biotinylated anti-rabbit IgG, diluted 1:500). This was followed by a 30 min wash in PB and incubation for 1 h in ExtrAvidin-horseradish peroxidase (Sigma; diluted 1:1000 in PBH). After three 30 min washes in PB, tissue was immersed for 10 min in a filtered solution of α -naphthol (0.05%), 0.1% ammonium carbonate and 0.003% H_2O_2 in distilled water, briefly rinsed with PB and then immersed in 0.1% pyronin B solution in PB for 7 min at room temperature. This procedure produces a pink precipitate in neurons containing 5-HT.⁶³ The sections were then mounted, cleared and coverslipped.

Counting of labelled cells

The amount of *c-fos* expression was quantified in 25 different brain regions or subregions with reference to the rat brain atlas of Paxinos and Watson.⁶² The regions counted



Fig. 2. Camera lucida drawing showing the widespread *c-fos* expression (black dots) in the dorsal and ventral striatum following 20 mg/kg MDMA. The coronal section is approximately 1.20 mm anterior to bregma according to the atlas of Paxinos and Watson.⁶² Equivalent camera lucida drawings of the *c-fos* expression induced by cocaine, amphetamine, morphine and nicotine can be seen in the recent article by Harlan and Garcia.³⁴

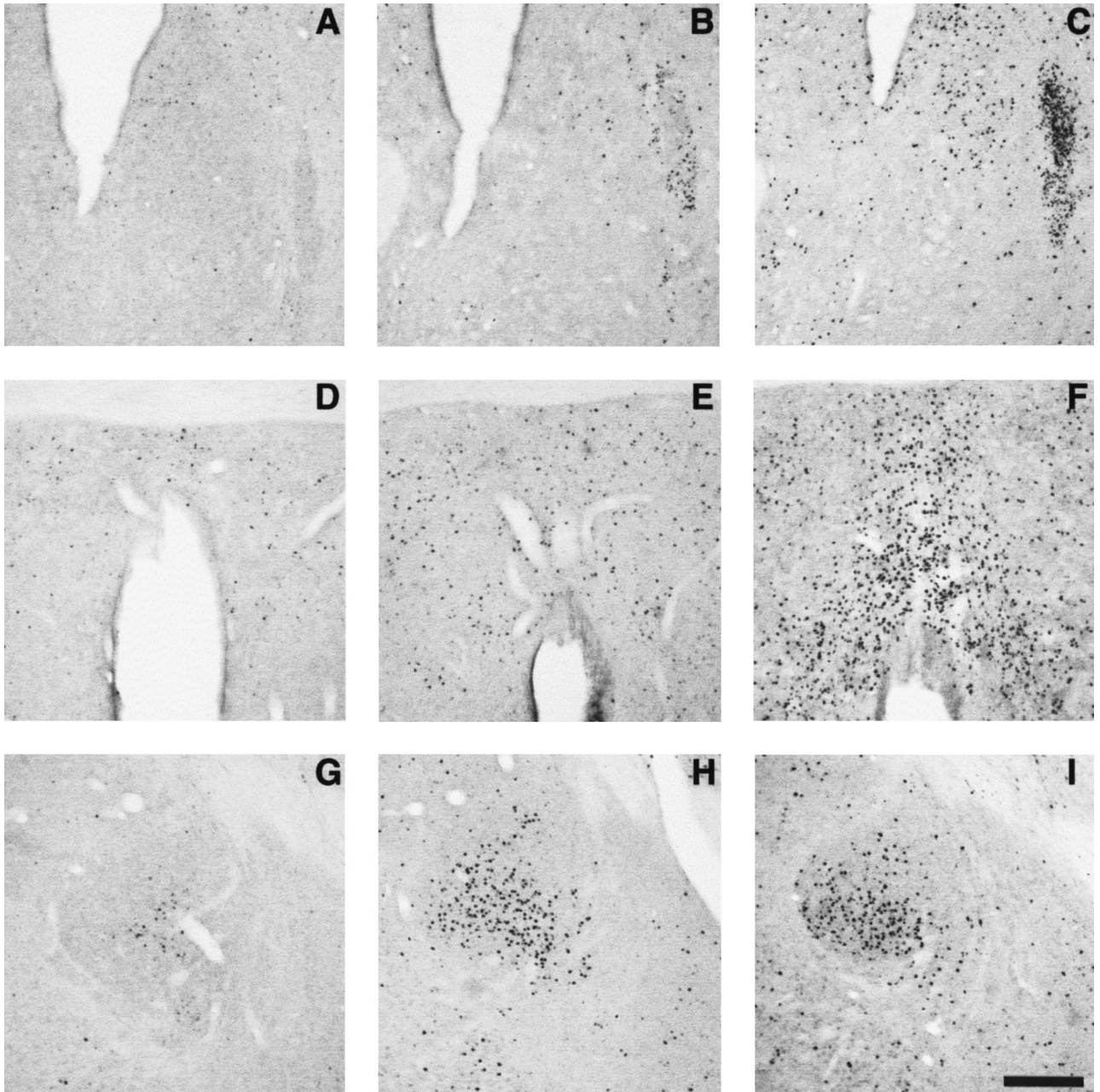


Fig. 3. The distribution of *c-fos* expression following saline, 5 mg/kg and 20 mg/kg MDMA, respectively, in the Islands of Calleja (A, B, C), median preoptic nucleus (D, E, F) and central nucleus of the amygdala (G, H, I). Scale bar = 250 μ m.

are listed in Table 1. As described previously,⁴⁰ quantification was performed manually by an observer who was blind to group assignment and using a graticule which equated to 0.5 mm² at a magnification of $\times 200$. This procedure was used for all structures except the islands of Calleja, median preoptic nucleus and the supraoptic nucleus where a $\times 400$ magnification (0.25 mm² area) was used since the regions were otherwise too small to fill the graticule. A neuron was classified as Fos-positive when the nucleus appeared round or oval, completely filled, and dark brown or black in colour.

Statistical analyses

Data from the 2 h locomotor activity test were divided

into 12 $\times$ 10 min bins for each rat with the total time spent moving during each bin (in seconds) calculated. Groups were compared on locomotor activity over the 12 bins using two-way ANOVA followed by a post hoc Tukey's test.

Data for *c-fos* counts for each brain region were compared across the three groups using one-way ANOVA followed by a post hoc Tukey's test.

RESULTS

Locomotor activity

Data for locomotor activity over the 2 h test

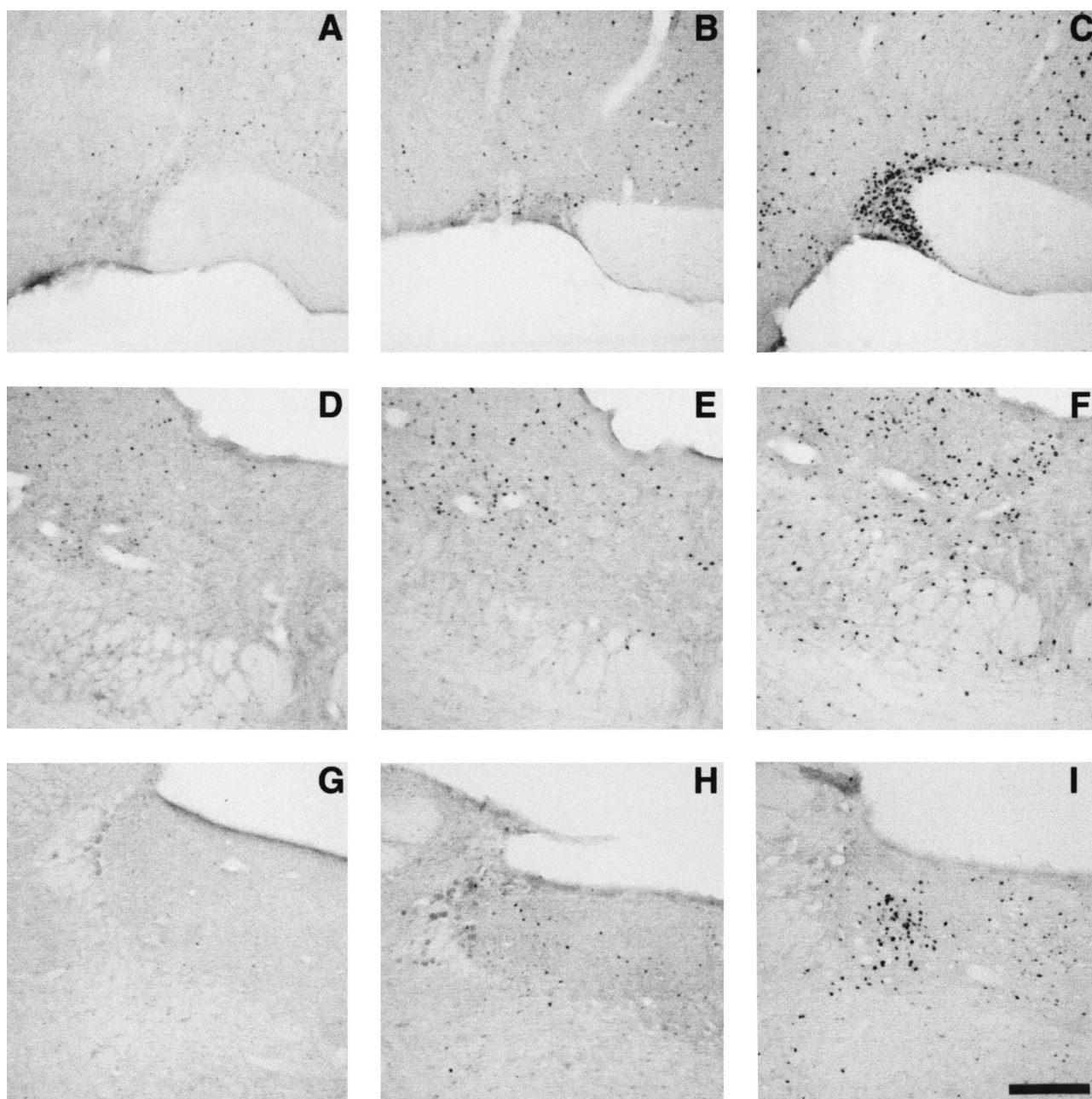


Fig. 4. The distribution of *c-fos* expression following saline, 5 mg/kg and 20 mg/kg MDMA, respectively, in the supraoptic nucleus (A, B, C), dorsal raphe (D, E, F) and Barrington's nucleus (G, H, I). Scale bar = 250 μ m.

period are shown in Fig. 1. Statistical analysis showed a significant effect of group ($F_{2,12} = 45.01$, $P < 0.001$), time ($F_{11,132} = 10.70$, $P < 0.001$), and a significant group by time interaction ($F_{22,132} = 1.85$, $P < 0.05$). Post hoc tests indicated greater overall activity in both MDMA groups compared to the vehicle group and greater activity in the 20 mg/kg MDMA group than the 5 mg/kg MDMA group. It can be seen from Fig. 1 that substantial hyperactivity was still present in MDMA-treated rats at 2 h following drug administration. Informal observations made immediately prior to perfusion indicated that rats given the 20 mg/kg dose exhibited many

signs of the "serotonin syndrome",³¹ including head weaving, flat body posture, forepaw treading and exophthalmos. In addition this group exhibited marked excitability, were wet under the chin and abdomen and were warm to the touch.

C-fos expression

MDMA caused dose-dependent increases in *c-fos* expression throughout the brain. Specific counts for 25 regions of interest are shown in Table 1, these regions representing the most salient locations of *c-fos* expression. While only one cortical area was

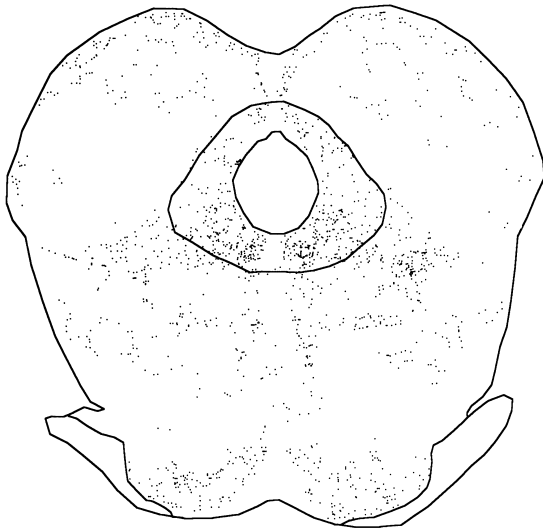


Fig. 5. Camera lucida drawing of a section approximately 8.30 mm posterior to bregma showing the *c-fos* expression (black dots) in the brainstem at the level of the dorsal raphe following 20 mg/kg MDMA.

counted (the medial prefrontal cortex), it can be noted that *c-fos* expression was observed through much of the cortex. In contrast, no *c-fos* expression was seen in any part of the hippocampus and so this region is not included in Table 1.

As Table 1 shows, there was very little *c-fos* expression in vehicle-treated rats, while the lower (5 mg/kg) dose of MDMA caused significant increases in *c-fos* expression in nine of the 25 brain regions of interest. The high (20 mg/kg) dose of MDMA showed significantly greater *c-fos* expression compared to the vehicle group in all regions counted. This group also showed significantly greater *c-fos* expression in all regions compared to the 5 mg/kg MDMA group, except in the central nucleus of the amygdala and the paraventricular thalamus where increases were equivalent with the two different doses.

Some of the most prominent *c-fos* expression was seen in the medial caudate–putamen. A camera lucida drawing of Fos-positive neurons in this and neighbouring regions is presented in Fig. 2. High levels were also present in the medial prefrontal cortex, nucleus accumbens, islands of Calleja, lateral septum, paraventricular thalamic nucleus, central nucleus of the amygdala, median preoptic nucleus and supraoptic nucleus (Figs 3, Fig. 4). In the brainstem, the most salient regions of *c-fos* expression were Barrington's nucleus, the Edinger–Westphal nucleus, the dorsal raphe and the pontine reticular nucleus oralis (Fig. 4). A camera lucida drawing of *c-fos* expression in a brainstem section at the level of the dorsal raphe is shown in Fig. 5. Very high levels of *c-fos* expression were also seen in the cerebellum, particularly in the flocculus.

C-fos expression in 5-hydroxytryptamine-positive neurons

Co-staining of Fos with 5-HT was seen in only a very small number of dorsal raphe, median raphe and raphe magnus neurons. Statistical analysis indicated that MDMA caused a significant increase in the number of double-labelled neurons in the dorsal raphe, from a mean of 0.60/0.5 mm² with vehicle to a mean of 2.0 with 5 mg/kg MDMA and 3.0 with 20 mg/kg MDMA. There was no significant increase in double-labelled cells in the median raphe or raphe magnus with either dose of MDMA with mean numbers remaining very modest (<1/0.5 mm²) regardless of drug condition.

DISCUSSION

MDMA produced a clear locomotor activation in rats as has been reported in several previous studies.^{12,30,43,54,66,78} Locomotor activation persisted for the duration of the 2 h post-drug activity test, agreeing with earlier findings that MDMA hyperactivity lasts up to 4 h.²⁹ The same two doses that caused pronounced locomotor hyperactivity, induced extensive *c-fos* expression in many regions of the forebrain and brainstem. While *c-fos* expression has previously been reported in the caudate–putamen, nucleus accumbens, olfactory tubercle and frontal cortex with doses of MDMA similar to those used here,^{22,35} the present study reports activation in a much greater range of structures, including regions of the hypothalamus, brainstem and cerebellum.

Presumably, much of the forebrain *c-fos* expression seen with MDMA is due to drug-induced 5-HT release causing enhanced activation of postsynaptic 5-HT receptors. Consistent with this, MDMA-induced *c-fos* expression was seen in many of the regions receiving dense 5-HT projections from the dorsal and median raphe. The pattern of forebrain *c-fos* expression seen with MDMA was reminiscent of the *c-fos* expression reported with other treatments that elevate brain 5-HT levels. Thus administration of the 5-HT precursor L-tryptophan in conjunction with the monoamine oxidase inhibitor tranylcypromine, causes elevated synaptic 5-HT and pronounced *c-fos* expression in the medial prefrontal cortex, nucleus accumbens, caudate–putamen, olfactory tubercle, median preoptic nuclei and lateral septum.⁵⁵ All of these regions also showed significantly elevated *c-fos* expression in the present study.

In another previous study, administration of the substituted amphetamine, *p*-chloroamphetamine (PCA), which increases 5-HT release in a similar manner to MDMA,⁵ elevated *c-fos* expression in the caudate–putamen, olfactory tubercle, islands of Calleja, nucleus accumbens and central nucleus of the amygdala.⁵⁶ Likewise, fenfluramine, an anorexic drug with 5-HT-releasing properties similar to PCA and MDMA,⁵ increased *c-fos* expression in the

frontal cortex, caudate–putamen, paraventricular nucleus of the hypothalamus, central nucleus of the amygdala and bed nucleus of the stria terminalis.^{41,45,68,70} Again, all of these structures showed increased *c-fos* expression in the present study following MDMA.

This raises the question as to whether MDMA has any unique properties with respect to the *c-fos* expression it induces. This is a difficult question to answer since the previous studies examining PCA, fenfluramine and L-tryptophan/tranylcypromine did not assess *c-fos* expression across the entire range of structures examined in the present study. Nonetheless, some distinctive effects of MDMA can be noted. Firstly, the strong activation of the supraoptic nucleus with MDMA was not seen with fenfluramine⁴¹ and was not reported upon with L-tryptophan/tranylcypromine or PCA.^{55,56} Activation of the dorsal raphe was also not seen with fenfluramine⁴¹ and was not reported upon with other treatments affecting 5-HT.^{55,56} Finally, very little *c-fos* expression was apparent in the nucleus accumbens following treatment with PCA,⁵⁶ yet robust expression was obtained in this region with MDMA.

One other important facet of MDMA action is its ability to increase synaptic levels of dopamine and noradrenaline.^{26,59,85} Thus at least some of the forebrain *c-fos* expression seen with MDMA may be attributable to increased dopamine or noradrenaline efflux. Consistent with this, the present study showed MDMA-induced *c-fos* expression in the ventral tegmental area, origin of the dopaminergic innervation of the forebrain. Further, a recent study has shown that MDMA induced *c-fos* expression in the caudate–putamen and olfactory tubercle is blocked by pre-treatment with the D1 dopamine receptor antagonist SCH 23390.³⁵ SCH 23390 has also been shown to block the striatal *c-fos* expression induced by fenfluramine.⁷⁰

3,4-Methylenedioxymethamphetamine “Ecstasy” effects on serotonergic neurons

The vast majority of 5-HT neurons projecting into the forebrain arise from the dorsal and median raphe nuclei located in the rostral pons.² The present study shows that MDMA significantly increases *c-fos* expression in both of these areas. However, double labelling with antibodies directed against serotonin indicated that most of the *c-fos* expression in the raphe nuclei was located in non-serotonergic neurons, which make up approximately 50% of raphe cells.³³ The absence of *c-fos* expression in serotonergic neurons in the raphe nuclei is consistent with electrophysiological evidence that MDMA inhibits firing of serotonergic raphe neurons via an indirect action of 5-HT_{1A} autoreceptors located on the soma and dendrites of these neurons.^{27,79} This activation of 5-HT_{1A} receptors causes hyperpolarization of raphe neurons, while *c-fos* is usually only

expressed after a depolarizing stimulus.³⁹ Because of this, *c-fos* expression would not be expected in the serotonergic neurons of the raphe nuclei. Future work will hopefully determine the neurochemical profile of the raphe neurons that are Fos positive.

The dorsal raphe showed a greater *c-fos* response to MDMA than the median raphe, with only the former structure showing significantly elevated *c-fos* expression with the lower 5 mg/kg dose of MDMA. This brings to mind suggestions that the 5-HT projections from the dorsal raphe may be more sensitive to the neurotoxic effects of MDMA than those from the median raphe.⁵⁰

Receptor subtypes involved in 3,4-methylenedioxymethamphetamine “Ecstasy”-induced *c-fos* expression

A question of obvious interest relates to the specific 5-HT receptors that may be driving the increased MDMA induced *c-fos* expression in various brain regions. This is a complex question, given the presence of at least 15 5-HT receptor subtypes,⁸² and a question that can perhaps only be properly answered using experiments in which selective 5-HT receptor antagonists are given in conjunction with MDMA. Nonetheless, some speculations can be made here concerning the likely receptor subtypes involved.

It seems unlikely that much of the *c-fos* expression can be due to activation of 5-HT₁ receptor subtypes since these all inhibit the production of cyclic AMP and are therefore more likely to suppress rather than promote *c-fos* expression.³⁹ Consistent with this, a recent study has shown that the 5-HT_{1A} receptor antagonist WAY 100,635 had no effect on the *c-fos* expression induced by fenfluramine.⁴¹ This may also help to explain the absence of *c-fos* expression in the hippocampus following MDMA. Despite receiving a dense serotonergic innervation, the hippocampus contains receptors that are predominantly of the 5HT_{1A} subtype, stimulation of which does not usually lead to *c-fos* expression.¹³ It remains possible however that some of the *c-fos* expression seen following MDMA may be indirectly mediated by 5-HT₁ receptor subtypes. It is known that 5-HT_{1A} agonists such as flesinoxan induce *c-fos* expression in a variety of sites including the central nucleus of the amygdala and paraventricular nucleus of the hypothalamus. However, this *c-fos* expression is thought to be transynaptically mediated, occurring downstream of the neurons on which the 5-HT_{1A} receptors are located.¹³

Some role for the 5-HT₂ receptor family seems likely given that stimulation of these receptors is linked to *c-fos* expression.³⁹ In agreement, a previous study found that the *c-fos* expression induced by PCA in the olfactory tubercle, islands of Calleja and caudate–putamen was attenuated by pretreatment with the 5-HT_{2A/2C} antagonist ritanserin.⁵⁶

Table 2. Possible neural substrates of 3,4-methylenedioxymethamphetamine "Ecstasy" effects

Effect	Possible neural substrate
ADH secretion/Hyponatremia	Supraoptic nucleus
Analgesia	Nucleus raphe magnus, periaqueductal gray
Anxiety	Bed nucleus of the stria terminalis, central nucleus of the amygdala, medial prefrontal cortex
Appetite suppression	Paraventricular nucleus of the hypothalamus
Body temperature changes	Median preoptic nucleus
Bruxism (jaw grinding)	Caudate-putamen, Pontine reticular nucleus oralis
Corticosterone secretion	Paraventricular nucleus of the hypothalamus
Euphoria/feelings of closeness	Nucleus accumbens, amygdala (?), cortex (?)
Hyperactivity	Nucleus accumbens, ventral tegmental area, caudate-putamen
Impaired co-ordination/dizziness/vertigo	Cerebellum
Sexual excitement	Median preoptic nucleus
Urination	Barrington's nucleus

Other 5-HT receptors that are probably involved in MDMA-induced *c-fos* expression are the 5-HT₄, 5-HT₆ and 5-HT₇ families since all are positively linked to cyclic AMP production (and hence *c-fos* expression) and all are widely distributed in the cortex, limbic system and brainstem.^{41,82} Development of selective antagonists for these receptor families will hopefully allow future studies to confirm a role for these receptors in mediating MDMA *c-fos* expression and behavioural effects.

Functional correlates of regional c-fos expression produced by 3,4-methylenedioxymethamphetamine "Ecstasy"

MDMA produces a wide range of physiological, behavioural and emotional changes including hyperactivity, hyperthermia, diaphoresis, dehydration, anorexia, analgesia, bruxism, and feelings of closeness towards others.^{46,64,77,83} Given the distinctive distribution of *c-fos* expression induced by MDMA, it is interesting to speculate on the possible neural basis for some of these diverse effects. A summary of possible functional correlates is given in Table 2.

MDMA caused a robust increase in *c-fos* expression in the nucleus accumbens septi (NAS) consistent with its well-documented ability to increase dopamine and serotonin efflux in this region.^{43,59} A role for the NAS in mediating MDMA-induced hyperactivity seems likely since 6-hydroxydopamine lesions of the NAS attenuate this effect.²⁹ Postsynaptic 5-HT_{1B} receptors have also been implicated in the hyperactivity produced by MDMA,^{28,31,66} possibly via an indirect effect on dopamine release in the NAS.¹¹ Dopamine release in the NAS has also been suggested to mediate the rewarding effects of drugs of abuse, although this theory is somewhat controversial.⁸⁶ Nonetheless, MDMA is rewarding to rats as shown by its ability to produce a conditioned place preference^{9,10} and to lower self-stimulation thresholds,⁴⁸ and the possibility remains that the NAS plays a critical role in this rewarding effect.

Interestingly, *c-fos* expression was much greater in the core than the shell region of the NAS (see

Table 1). This is consistent with findings that the shell is less susceptible to MDMA-mediated inhibition of glutamate evoked firing, whilst the core and caudate-putamen are equally susceptible to this effect.⁸⁴ This may in turn be linked to lesser release of 5-HT by the large diameter terminals in the shell region, and a concomitant smaller release of dopamine.⁸⁵

The hypothalamic nuclei including the median preoptic nuclei (MPO) and supraoptic nuclei showed pronounced *c-fos* expression. The paraventricular nucleus of the hypothalamus (PVN) also showed a marked increase in *c-fos* expression although this effect was more variable across rats. The MPO expresses *c-fos* following exposure to a hot environment,⁷² and it is well known that MDMA causes hyperthermia when given at ambient temperatures similar to those used in the present study.^{17,58} This hyperthermic effect of MDMA may be a peripheral effect mediated by an increase in myoplasmic calcium. Thus MDMA increases myoplasmic calcium in muscle cells *in vitro* and this effect is reversed by administration of Dantrolene, which also reverses MDMA-induced hyperthermia.^{19,32} However, central effects may also play a role in MDMA-induced hyperthermia since treatments that elevate levels of 5-HT in the MPO cause hyperthermia in rats.⁴⁹ Thus, it may be that the MPO not only responds to peripherally-mediated MDMA hyperthermia but also contributes to the genesis of hyperthermia in its own right.

Other evidence has consistently implicated the MPO and adjacent medial preoptic area in the control of sexual behaviour, with *c-fos* expression prominent in this region following copulation and ejaculation.⁷ Thus MDMA-induced *c-fos* expression in this region might also be reflective of the modulation of sexual behaviour by the drug. MDMA can produce sexual arousal in humans,²¹ and is known to produce ejaculation in rats when given acutely in moderate doses.⁸ On the other hand, high repeated doses of MDMA have been associated with a disruption of normal sexual behaviour in male rats.²⁰

The supraoptic nuclei (SON) have been

implicated in the control of serum osmolality via an effect on antidiuretic hormone (ADH) release.⁸⁰ Interestingly, MDMA has recently been found to induce ADH secretion in humans.³⁶ Consequently the very high concentration of *c-fos* expression in the SON could indicate the mechanism underlying the potent effect of MDMA on ADH release. A number of deaths from MDMA use have been attributed to hyponatremia^{37,76} arising from overhydration. While this has often been attributed to overzealous water consumption in some MDMA users, it might also reflect a syndrome of inappropriate ADH secretion mediated by activation of the SON.³⁸

The pronounced *c-fos* expression seen in Barrington's nucleus may also have implications with respect to altered fluid balance after MDMA. For many decades, Barrington's nucleus has been recognized as the key brainstem region for the control of micturition.^{3,69,71} Thus, the *c-fos* expression caused by MDMA in this region suggests a possible modulatory effect of MDMA on urinary output. Indeed, in one study MDMA was found to increase urination in rats⁸ while a recent study in humans suggests a similar effect, albeit in some but not all subjects.⁸³ Presumably, the need to urinate may result from the increased fluid retention provoked by the effect of MDMA on ADH secretion.

With respect to the cerebellum, the dense *c-fos* expression caused by MDMA in this region suggests some major effect on motor co-ordination or balance. Consistent with this, a recent study has shown that the majority of human subjects given MDMA for the first time reported impaired balance and gait with substantial numbers also reporting dizziness or vertigo.⁸³ In this recent study, another prominent effect, seen in nearly every subject given MDMA, was jaw clenching (bruxism). This effect is consistent with the robust *c-fos* expression induced by MDMA in the pontine reticular nucleus oralis (PNO), a region known to be involved in several functions including control of masticatory muscles.²⁴ The dense *c-fos* expression in the caudate-putamen could also be linked to bruxism, since infusions of dopaminergic or serotonergic agonists into the ventrolateral or ventromedial caudate is known to induce stereotyped oral behaviour.⁸⁸

MDMA also induced substantial *c-fos* expression in brainstem regions involved in analgesia. The periaqueductal gray and nucleus raphe magnus are both components of a descending inhibitory network that modulates nociceptive neurotransmission in the spinal cord. The *c-fos* expression seen in both of these structures is consistent with findings that MDMA administration induces marked analgesia in the rat.¹⁴

The central nucleus of the amygdala (CEA) was the only amygdala region to express significant amounts of *c-fos* following MDMA. The central nucleus is a region that is traditionally associated with fear and anxiety and which exerts control

over corticosterone secretion via a pathway involving the bed nucleus of the stria terminalis (BNST) and the PVN.^{18,44} Consistent with an excitatory effect on this pathway, MDMA caused *c-fos* expression in the CEA, BNST and PVN and has been shown in other studies to induce corticosterone release.⁵⁸ The medial prefrontal cortex (MPC) is also a region associated with fear and anxiety^{23,42} and this region also showed profound increases in *c-fos* expression with MDMA in the present study. Whether MDMA induced *c-fos* expression in amygdala and prefrontal regions is in any way correlated with fear and anxiety is uncertain. MDMA has been reported to occasionally induce panic attacks in humans,⁵³ and other evidence suggests anxiogenic effects of the drug in rats.⁶ Nonetheless, MDMA has also been shown to produce rewarding and anxiolytic effects in rats^{9,10,47,48,87} and the overwhelming effect of the drug in humans seems to be euphoric rather than anxiogenic. This suggests that MDMA may have the capacity to induce both euphoria and anxiety, with contextual or personality variables perhaps determining the outcome of the drug experience from one situation or individual to the next.

The final, and perhaps most mysterious question then relates to the neural substrates underlying the powerful positive feelings of "Ecstasy" produced by MDMA. As well as having a role in anxiety, many recent studies suggest that the amygdala and prefrontal cortex play key roles in the detection, formulation and interpretation of emotions in general.^{1,4,57,65} Thus MDMA activation of these two regions might be linked to the unique feelings of empathy and enhanced communication with others that the drug is reported to bring.⁴⁶

CONCLUSIONS

The present study demonstrates that MDMA induces a widespread and distinctive activation of many brain regions as shown by the technique of *c-fos* immunohistochemistry. Many of the functional effects of MDMA can be traced to a specific action on brain regions, with the caveat that *c-fos* immunohistochemistry probably only indicates a subset of the regions that MDMA influences. Functional aspects of MDMA-induced *c-fos* expression in the nucleus accumbens, median preoptic nucleus, supraoptic nuclei, cerebellum, raphe magnus and Barrington's nucleus can be readily speculated upon. Yet the substrates underlying the most desirable effects of MDMA reported by humans, such as increased confidence and feelings of closeness to others, remain rather mysterious.

Acknowledgements—Research supported by a NH and MRC project grant to G.E.H and I.S.M. We are grateful to NIDA for the generous gift of MDMA.

REFERENCES

- Adolphs R., Tranel D. and Damasio A. R. (1998) The human amygdala in social judgment. *Nature* **393**, 470–474.
- Azmitia E. C. and Segal M. (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. comp. Neurol.* **179**, 641–667.
- Barrington F. J. F. (1925) The effect of lesions of the hind- and mid-brain on micturition in the cat. *Q. Jl exp. Physiol. cog. Med.* **15**, 81–102.
- Bechara A., Tranel D., Damasio H. and Damasio A. R. (1996) Failure to respond autonomically to anticipated future outcomes following damage to prefrontal cortex. *Cerebral Cortex* **6**, 215–225.
- Berger U. V., Gu X. F. and Azmitia E. C. (1992) The substituted amphetamines 3,4-methylenedioxymethamphetamine, methamphetamine, p-chloroamphetamine and fenfluramine induce 5-hydroxytryptamine release via a common mechanism blocked by fluoxetine and cocaine. *Eur. J. Pharmac.* **215**, 153–160.
- Bhattacharya S. K., Bhattacharya A. and Ghosal S. (1998) Anxiogenic activity of methylenedioxymethamphetamine (Ecstasy)—an experimental study. *Biogenic Amines* **14**, 217–237.
- Bialy M. and Kaczmarek L. (1996) c-Fos expression as a tool to search for the neurobiological base of the sexual behaviour of males. *Acta neurobiol. exp.* **56**, 567–577.
- Bilsky E. J., Hubbell C. L., Delconte J. D. and Reid L. D. (1991) MDMA produces a conditioned place preference and elicits ejaculation in male rats: a modulatory role for the endogenous opioids. *Pharmac. Biochem. Behav.* **40**, 443–447.
- Bilsky E. J., Hui Y. Z., Hubbell C. L. and Reid L. D. (1990) Methylenedioxymethamphetamine's capacity to establish place preferences and modify intake of an alcoholic beverage. *Pharmac. Biochem. Behav.* **37**, 633–638.
- Bilsky E. J., Montegut M. J., Nichols M. L. and Reid L. D. (1998) CGS 10746B, a novel dopamine release inhibitor, blocks the establishment of cocaine and MDMA conditioned place preferences. *Pharmac. Biochem. Behav.* **59**, 215–220.
- Boulenguez P., Rawlins J. N., Chauveau J., Joseph M. H., Mitchell S. N. and Gray J. A. (1996) Modulation of dopamine release in the nucleus accumbens by 5-HT_{1B} agonists: involvement of the hippocampo-accumbens pathway. *Neuropharmacology* **35**, 1521–1529.
- Callaway C. W., Wing L. L. and Geyer M. A. (1990) Serotonin release contributes to the locomotor stimulant effects of 3,4-methylenedioxymethamphetamine in rats. *J. Pharmac. exp. Ther.* **254**, 456–464.
- Compaan J. C., Groenink L., van der Gugten J., Maes R. A. and Olivier B. (1996) 5-HT_{1A} receptor agonist flesinoxan enhances Fos immunoreactivity in rat central amygdala, bed nucleus of the stria terminalis and hypothalamus. *Eur. J. Neurosci.* **8**, 2340–2347.
- Crisp T., Stafinsky J. L., Boja J. W. and Schechter M. D. (1989) The antinociceptive effects of 3,4-methylenedioxymethamphetamine (MDMA) in the rat. *Pharmac. Biochem. Behav.* **34**, 497–501.
- Curran H. V. and Travill R. A. (1997) Mood and cognitive effects of $\pm$ 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy'): week-end 'high' followed by mid-week low. *Addiction* **92**, 821–831.
- Curran T. and Morgan J. I. (1995) Fos: an immediate-early transcription factor in neurons. *J. Neurobiol.* **26**, 403–412.
- Dafters R. I. (1995) Hyperthermia following MDMA administration in rats: effects of ambient temperature, water consumption, and chronic dosing. *Physiol. Behav.* **58**, 877–882.
- Davis M., Walker D. L. and Lee Y. (1997) Amygdala and bed nucleus of the stria terminalis: differential roles in fear and anxiety measured with the acoustic startle reflex. *Phil. Trans R. Soc.* **352**, 1675–1687.
- Denborough M. A. and Hopkinson K. C. (1997) Dantrolene and ecstasy. *Med. J. Aus.* **166**, 165–166.
- Dornan W. A., Katz J. L. and Ricaurte G. A. (1991) The effects of repeated administration of MDMA on the expression of sexual behavior in the male rat. *Pharmac. Biochem. Behav.* **39**, 813–816.
- Downing J. (1986) The psychological and physiological effects of MDMA on normal volunteers. *J. psychoact. Drugs* **18**, 335–340.
- Dragunow M., Logan B. and Lavery R. (1991) 3,4-Methylenedioxymethamphetamine induces Fos-like proteins in rat basal ganglia: reversal with MK 801. *Eur. J. Pharmac.* **206**, 255–258.
- Duncan G. E., Knapp D. J., Johnson K. B. and Breese G. R. (1996) Functional classification of antidepressants based on antagonism of swim stress-induced fos-like immunoreactivity. *J. Pharmac. exp. Ther.* **277**, 1076–1089.
- Fay R. A. and Norgren R. (1997) Identification of rat brainstem multisynaptic connections to the oral motor nuclei using pseudorabies virus. 1 Masticatory muscle motor systems. *Brain Res. Rev.* **25**, 255–275.
- Fischer C., Hatzidimitriou G., Wlos J., Katz J. and Ricaurte G. (1995) Reorganization of ascending 5-HT axon projections in animals previously exposed to the recreational drug ($\pm$) 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *J. Neurosci.* **15**, 5476–5485.
- Fitzgerald J. L. and Reid J. J. (1990) Effects of methylenedioxymethamphetamine on the release of monoamines from rat brain slices. *Eur. J. Pharmac.* **191**, 217–220.
- Gartside S. E., McQuade R. and Sharp T. (1997) Acute effects of 3,4-methylenedioxymethamphetamine (MDMA) on 5-HT cell firing and release: comparison between dorsal and median raphe 5-HT systems. *Neuropharmacology* **36**, 1697–1703.
- Geyer M. A. (1996) Serotonergic functions in arousal and motor activity. *Behav. Brain Res.* **73**, 31–35.
- Gold L. H., Hubner C. B. and Koob G. F. (1989) A role for the mesolimbic dopamine system in the psychostimulant actions of MDMA. *Psychopharmacology* **99**, 40–47.
- Gold L. H. and Koob G. F. (1988) Methysergide potentiates the hyperactivity produced by MDMA in rats. *Pharmac. Biochem. Behav.* **29**, 645–648.
- Green A. R. and Backus L. I. (1990) Animal models of serotonin behavior. *Ann. N. Y. Acad. Sci.* **600**, 237–248.
- Green A. R., Cross A. J. and Goodwin G. M. (1995) Review of the pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy"). *Psychopharmacology* **119**, 247–260.
- Halliday G., Harding A. and Paxinos G. (1995) Serotonin and tachykinin systems. In *The Rat Nervous System* (ed. Paxinos G.), pp. 929–971. Academic, New York.
- Harlan R. E. and Garcia M. M. (1998) Drugs of abuse and immediate-early genes in the forebrain. *Molec. Neurobiol.* **16**, 221–267.
- Hashimoto K., Tomitaka S., Narita N., Minabe Y. and Iyo M. (1997) Induction of Fos protein by 3,4-methylenedioxymethamphetamine (Ecstasy) in rat brain: regional difference in pharmacological manipulation. *Addiction Biol.* **2**, 317–326.
- Henry J. A., Fallon J. K., Kicman A. T., Hutt A. J., Cowan D. A. and Forsling M. (1998) Low-dose MDMA ("ecstasy") induces vasopressin secretion. *Lancet* **351**, 1784.

37. Henry J. A., Jeffreys K. J. and Dawling S. (1992) Toxicity and deaths from 3,4-methylenedioxymethamphetamine ("ecstasy"). *Lancet* **340**, 384–387.
38. Holden R. and Jackson M. A. (1996) Near-fatal hyponatraemic coma due to vasopressin over-secretion after "ecstasy" (3,4-MDMA). *Lancet* **347**, 1052.
39. Hughes P. and Dragunow M. (1995) Induction of immediate-early genes and the control of neurotransmitter-regulated gene expression within the nervous system. *Pharmac. Rev.* **47**, 133–178.
40. Hunt G. E. and McGregor I. S. (1998) Rewarding brain stimulation induces only sparse fos-like immunoreactivity in dopaminergic neurons. *Neuroscience* **83**, 501–515.
41. Javed A., Van de Kar L. D. and Gray T. S. (1998) The 5-HT_{1A} and 5-HT_{2A/2C} receptor antagonists WAY-100635 and ritanserin do not attenuate D-fenfluramine-induced Fos expression in the brain. *Brain Res.* **791**, 67–74.
42. Jinks A. L. and McGregor I. S. (1997) Modulation of anxiety-related behaviours following lesions of the prelimbic or infralimbic cortex in the rat. *Brain Res.* **772**, 181–190.
43. Kalivas P. W., Duffy P. and White S. R. (1998) MDMA elicits behavioral and neurochemical sensitization in rats. *Neuropsychopharmacology* **18**, 469–479.
44. Ledoux J. E. and Muller J. (1997) Emotional memory and psychopathology. *Phil. Trans R. Soc.* **352**, 1719–1726.
45. Li B. H. and Rowland N. E. (1996) Effect of chronic dexfenfluramine on Fos in rat brain. *Brain Res.* **728**, 188–192.
46. Liester M. B., Grob C. S., Bravo G. L. and Walsh R. N. (1992) Phenomenology and sequelae of 3,4-methylenedioxymethamphetamine use. *J. nerv. mental Disease* **180**, 345–352.
47. Lin H. Q., Burden P. M., Christie M. J. and Johnston G. A. R. (1999) The anxiogenic and anxiolytic effects of MDMA on mice in the elevated plus maze: a comparison with amphetamine. *Pharmacol. Biochem. Behav.* **62**, 403–408.
48. Lin H. Q., Jackson D. M., Atrens D. M., Christie M. J. and McGregor I. S. (1997) Serotonergic modulation of 3,4-methylenedioxymethamphetamine (MDMA)-elicited reduction of response rate but not rewarding threshold in accumbal self-stimulation. *Brain Res.* **744**, 351–357.
49. Lin M. T., Tsay H. J., Su W. H. and Chueh F. Y. (1998) Changes in extracellular serotonin in rat hypothalamus affect thermoregulatory function. *Am. J. Physiol.* **274**, R1260–R1267.
50. Mamounas L. A., Mullen C. A., O'Hearn E. and Molliver M. E. (1991) Dual serotonergic projections to forebrain in the rat: morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives. *J. comp. Neurol.* **314**, 558–586.
51. McCann U. D. and Ricaurte G. A. (1992) MDMA ("ecstasy") and panic disorder: induction by a single dose. *Biol. Psychiat.* **32**, 950–953.
52. McCann U. D., Szabo Z., Scheffel U., Dannals R. F. and Ricaurte G. A. (1998) Positron emission tomographic evidence of toxic effect of MDMA (ecstasy) on brain serotonin neurons in human beings. *Lancet* **352**, 1433–1437.
53. McGuire P. K., Cope H. and Fahy T. A. (1994) Diversity of psychopathology associated with use of 3,4-methylenedioxymethamphetamine ('Ecstasy'). *Br. J. Psychiat.* **165**, 391–395.
54. McNamara M. G., Kelly J. P. and Leonard B. E. (1995) Some behavioural and neurochemical aspects of subacute ($\pm$)3,4-methylenedioxymethamphetamine administration in rats. *Pharmac. Biochem. Behav.* **52**, 479–484.
55. Moorman J. M., Jackson A., Grahame-Smith D. G. and Leslie R. A. (1995) Induction of c-fos in rat forebrain by pharmacological manipulation of 5-hydroxytryptamine levels. *Neuroscience* **68**, 1089–1096.
56. Moorman J. M. and Leslie R. A. (1996) P-chloroamphetamine induces c-fos in rat brain: a study of serotonin_{2A/2C} receptor function. *Neuroscience* **72**, 129–139.
57. Morris J. S., Frith C. D., Perrett D. I., Rowland D., Young A. W., Calder A. J. and Dolan R. J. (1996) A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature* **383**, 812–815.
58. Nash J. F. Jr., Meltzer H. Y. and Gudelsky G. A. (1988) Elevation of serum prolactin and corticosterone concentrations in the rat after the administration of 3,4-methylenedioxymethamphetamine. *J. Pharmac. exp. Ther.* **245**, 873–879.
59. Obradovic T., Imel K. M. and White S. R. (1998) Repeated exposure to methylenedioxymethamphetamine (MDMA) alters nucleus accumbens neuronal responses to dopamine and serotonin. *Brain Res.* **785**, 1–9.
60. O'Hearn E., Battaglia G., De Souza E. B., Kuhar M. J. and Molliver M. E. (1988) Methylenedioxymethamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. *J. Neurosci.* **8**, 2788–2803.
61. Parrott A. C., Lees A., Garnham N. J., Jones M. and Wesnes K. (1998) Cognitive performance in recreational users of MDMA or 'ecstasy': evidence for memory deficits. *J. Psychopharmac.* **12**, 79–83.
62. Paxinos G. and Watson, C. (1997) *The Rat Brain in Stereotaxic Coordinates*, 3rd edn. Academic, Sydney.
63. Peng Z. C., Chen S. and Bentivoglio M. (1995) A sensitive double immunostaining protocol for Fos-immunoreactive neurons. *Brain Res. Bull.* **36**, 101–105.
64. Peroutka S. J., Newman H. and Harris H. (1988) Subjective effects of 3,4-methylenedioxymethamphetamine in recreational users. *Neuropsychopharmacology* **1**, 273–277.
65. Pitkanen A., Savander V. and LeDoux J. E. (1997) Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. *Trends Neurosci.* **20**, 517–523.
66. Rempel N. L., Callaway C. W. and Geyer M. A. (1993) Serotonin_{1B} receptor activation mimics behavioral effects of pre-synaptic serotonin release. *Neuropsychopharmacology* **8**, 201–211.
67. Ricaurte G. A. and McCann U. D. (1992) Neurotoxic amphetamine analogues: effects in monkeys and implications for humans. *Ann. N. Y. Acad. Sci.* **648**, 371–382.
68. Richard D., Rivest S. and Rivier C. (1992) The 5-hydroxytryptamine agonist fenfluramine increases Fos-like immunoreactivity in the brain. *Brain Res.* **594**, 131–137.
69. Rizvi T. A., Murphy A. Z., Ennis M., Aston-Jones G. and Shipley M. T. (1998) Fos expression in rat pontine tegmental neurons following activation of the medial preoptic area. *Brain Res.* **789**, 256–262.
70. Rouillard C., Bovetto S., Gervais J. and Richard D. (1996) Fenfluramine-induced activation of the immediate-early gene c-fos in the striatum: possible interaction between serotonin and dopamine. *Molec. Brain Res.* **37**, 105–115.
71. Satoh K., Shimizu N., Toyama M. and Maeda T. (1978) Localization of the micturition reflex centre in the dorsolateral pontine tegmentum in the rat. *Neurosci. Lett.* **8**, 27–35.
72. Scammell T. E., Price K. J. and Sagar S. M. (1993) Hyperthermia induces c-fos expression in the preoptic area. *Brain Res.* **618**, 303–307.
73. Scanzello C. R., Hatzidimitriou G., Martello A. L., Katz J. L. and Ricaurte G. A. (1993) Serotonergic recovery after ($\pm$)3,4-

- (methylenedioxy) methamphetamine injury: observations in rats. *J. Pharmac. exp. Ther.* **264**, 1484–1491.
74. Schmidt C. J., Black C. K. and Taylor V. L. (1990) Antagonism of the neurotoxicity due to a single administration of methylenedioxymethamphetamine. *Eur. J. Pharmac.* **181**, 59–70.
 75. Schmidt C. J., Levin J. A. and Lovenberg W. (1987) *In vitro* and *in vivo* neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. *Biochem. Pharmac.* **36**, 747–755.
 76. Schwartz R. H. and Miller N. S. (1997) MDMA (ecstasy) and the rave: a review. *Pediatrics* **100**, 705–708.
 77. Solowij N., Hall W. and Lee N. (1992) Recreational MDMA use in Sydney: a profile of 'Ecstasy' users and their experiences with the drug. *Br. J. Addict.* **87**, 1161–1172.
 78. Spanos L. J. and Yamamoto B. K. (1989) Acute and subchronic effects of methylenedioxymethamphetamine [(±)MDMA] on locomotion and serotonin syndrome behavior in the rat. *Pharmac. Biochem. Behav.* **32**, 835–840.
 79. Sprouse J. S., Bradberry C. W., Roth R. H. and Aghajanian G. K. (1990) 3,4-Methylenedioxymethamphetamine-induced release of serotonin and inhibition of dorsal raphe cell firing: potentiation by L-tryptophan. *Eur. J. Pharmac.* **178**, 313–320.
 80. Swaab D. F., Hofman M. A., Lucassen P. J., Purba J. S., Raadsheer F. C. and Van de Nes J. A. (1993) Functional neuroanatomy and neuropathology of the human hypothalamus. *Anat. Embryol.* **187**, 317–330.
 81. Topple A. N., Hunt G. E. and McGregor I. S. (1998) Possible neural substrates of beer-craving in rats. *Neurosci. Lett.* **252**, 99–102.
 82. Uphouse L. (1997) Multiple serotonin receptors: too many, not enough, or just the right number? *Neurosci. biobehav. Rev.* **21**, 679–698.
 83. Vollenweider F. X., Gamma A., Liechti M. and Huber T. (1998) Psychological and cardiovascular effects and short-term sequelae of MDMA ("Ecstasy") in MDMA-naïve healthy volunteers. *Neuropsychopharmacology* **19**, 241–251.
 84. White S. R., Harris G. C., Imel K. M. and Wheaton M. J. (1995) Inhibitory effects of dopamine and methylenedioxymethamphetamine (MDMA) on glutamate-evoked firing of nucleus accumbens and caudate/putamen cells are enhanced following cocaine self-administration. *Brain Res.* **681**, 167–176.
 85. White S. R., Obradovic T., Imel K. M. and Wheaton M. J. (1996) The effects of methylenedioxymethamphetamine (MDMA, "Ecstasy") on monoaminergic neurotransmission in the central nervous system. *Prog. Neurobiol.* **49**, 455–479.
 86. Wickelgren I. (1997) Getting the brain's attention. *Science* **278**, 35–37.
 87. Winslow J. T. and Insel T. R. (1990) Serotonergic modulation of rat pup ultrasonic vocal development: studies with 3,4-methylenedioxymethamphetamine. *J. Pharmac. exp. Ther.* **254**, 212–220.
 88. Yeghiayan S. K., Kelley A. E., Kula N. S., Campbell A. and Baldessarini R. J. (1997) Role of dopamine in behavioral effects of serotonin microinjected into rat striatum. *Pharmac. Biochem. Behav.* **56**, 251–259.

(Accepted 18 January 1999)